ENZYMATIC DELIGNIFICATION OF WOODY AND NONWOODY PLANT FEEDSTOCKS

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Lignocellulosic crops represent a renewable resource that has been well integrated into various industrial processes. This lignocellulosic biomass serves as feedstock for the lumber and pulp and paper industries. On the other hand, the use of lignocellulosic biomass has attracted increased interest for the production of biofuels. Ethanol world production from starch or sugar-producing crops is still much higher than that from cellulose. However, the potential for bioenergy production from lignocellulosic biomass sourced from grasses, crop residues and wood far exceeds starch or sugar crops and can be sourced from a much greater proportion of the available land (Seguin, 2011). Lignin removal is an important technical issue for paper manufacturing and is also the key challenge for the conversion of lignocellulosic feedstock into liquid transportation fuels such as ethanol. Biofuel production from lignocellulosic material requires deconstruction of the cell-wall matrix into individual polymers, and then of the carbohydrate polymers into their constitutive elements (monomeric sugars) to be fermented into ethanol. If this drawback is solved, low cost lignocellulosic materials can be the source of choice to obtain renewable fuels in the future. Biomass recalcitrance towards enzymatic hydrolysis is correlated to both the content and composition of lignin (Studer et al., 2011) that acts as a hardly-degradable glue between the cell-wall polysaccharides. Several approaches including physical, chemical and biological pretreatments, or combination of these processes, are being studied for deconstructing the lignocellulosic biomass and removing lignin (Alvira et al., 2010). Most biological pretreatments suggested to date for delignifying lignocellulosic materials employ lignin-degrading fungi, mainly belonging to the group of white-rot basidiomycetes but such pretreatments require long application periods and necessarily consume a fraction of the plant polysaccharides.

Laccases are multicopper oxidases that oxidize substituted phenols using molecular oxygen as the final electron acceptor, and have been object of high interest for the development of environmentally-benign technologies. The direct action of laccases on lignin is in principle restricted to the phenolic units that only represent a small percentage of the total polymer, a fact that limits its biotechnological application.

However, the interest on laccases as industrial biocatalysts steadily increased after discovering the effect of some synthetic compounds acting as electron carriers between the enzyme and the final substrate, 1-hydroxybenzotriazole (HBT) being among the most efficient ones. In this way, the action of laccase is expanded to non-phenolic substrates, which are oxidized by the stable mediator radicals. Since then, a variety of studies have confirmed the potential of the so-called laccase-mediator system mainly for paper pulp delignification but also for other applications.

The present study shows the ability of a laccase to remove lignin and make cellulose accessible to hydrolysis for conversion to fuels, when applied on the lignocellulosic biomass in combination with a redox mediator. Two lignocellulosic feedstocks, eucalypt (*Eucalyptus globulus*) and Elephant grass (*Pennisetum purpureum*), were selected as representative for rapid growth and high biomass producing woody and nonwoody plant species, respectively. The modification of lignin in the pretreated lignocellulosic materials was analyzed by two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy of the whole sample at the gel state, after swelling in dimethylsulfoxide-d_6 (Kim et al., 2008; Rencoret et al., 2009). This is a new and promising analytical methodology for the *in situ* analysis of lignin in plant materials, without the need of its time-consuming and low-yield isolation, of interest for enzymatic delignification studies. In addition to lignin removal, the effect of the enzymatic treatments on sugar and ethanol yield from the two pretreated lignocellulosic materials was also assessed.

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Alvira, P., Tomas-Pejo, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresource Technology 101, 4851-4861.

Kim, H., Ralph, J., Akiyama, T., 2008. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d_6. Bioenerg. Res. 1, 56-66.

Rencoret, J., Marques, G., Gutiérrez, A., Nieto, L., Santos, I., Jiménez-Barbero, J., Martínez, A.T., del Río, J.C., 2009. HSQC-NMR analysis of lignin in woody (*Eucalyptus globulus* and *Picea abies*) and non-woody (*Agave sisalana)* ball-milled plant materials at the gel state. Holzforschung 63, 691-698.

Seguin, A., 2011. How could forest trees play an important role as feedstock for bioenergy production? Curr. Opin. Environ. Sustain. 3, 90-94.

Studer, M.H., DeMartini, J.D., Davis, M.F., Sykes, R.W., Davison, B., Keller, M., Tuskan, G.A., Wyman, C.E., 2011. Lignin content in natural *Populus* variants affects sugar release. Proc. Natl. Acad. Sci. USA 108, 6300-6305.